Lung dust content in idiopathic pulmonary fibrosis: a study with scanning electron microscopy and energy dispersive *x* ray analysis

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Abstract

Examination with an optical microscope and polarised light is not sensitive enough to detect low diameter asbestos fibres. This limitation implies that some cases of asbestosis can be erroneously diagnosed as idiopathic pulmonary fibrosis (IPF) if asbestos bodies are not found in the standard examination of abnormal tissue. To determine whether IPF is overdiagnosed, a study was carried out with scanning electron microscopy (SEM) and energy dispersive *x* ray analysis (EDXA) on 25 samples previously diagnosed as IPF at the standard examination. Scanning electron microscopy will show the presence of low diameter fibres in the lung without tissue destruction, and these fibres can be identified using EDXA. The quantitative and qualitative results for lung tissue from patients diagnosed as having IPF were compared with the results of the examination of 25 samples of normal lung. Most of the samples from patients diagnosed as having IPF showed only occasional inorganic particles (<10 particles/SEM field at 160×), results equivalent to the results obtained in normal lung. Two cases of IPF, however, showed innumerable asbestos fibres (>100 fibres/SEM field). One of these two patients had an antecedent of brief exposure to asbestos. No environmental antecedent was found in the second patient. Asbestosis was the final diagnosis for these two patients. The examination of inorganic particles in normal lungs showed mainly non-fibrous silicates (61·4%) and particles of heavy elements (34·9%). Only one asbestos fibre was found (0·9%). It is concluded that standard pathological techniques overdiagnose IPF in a few cases in which asbestos bodies are not found with the optical microscope.

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A lung sample showing pulmonary fibrosis and inorganic particles allows the diagnosis of pneumoconiosis. Absence of inorganic particles in a fibrotic lung sample of a patient with diffuse interstitial lung disease, if no other cause is determined, usually gives a diagnosis of idiopathic pulmonary fibrosis (IPF). It is well known, however, that examination with an optical microscope and polarised light is not sensitive enough to detect low diameter inorganic particles, especially asbestos fibres. For lung samples from patients previously diagnosed by optical microscopy as having IPF, an examination using more sensitive techniques could find that some of the samples contain enough asbestos fibres to diagnose asbestosis.

Advances in mineralogical analysis have enabled its application in pneumology during the last two decades.[1-3] Among the techniques applicable to organic matter, scanning electron microscopy (SEM) with energy dispersive *x* ray analysis (EDXA) is especially useful.[4,5] Inorganic particles in the lung are easily identified if secondary and backscattered imaging are used (spot 0·125 μm; maximum resolution power 150 Å). The atomic composition of every inorganic particle visualised can be identified using EDXA. If the area studied in all the samples is predetermined, a quantitative and qualitative examination can be done.

We studied 25 samples with a previous pathological diagnosis of IPF and 25 samples of normal lung, and determined the number of inorganic particles seen in the area visualised with SEM at 160 × (278 300 μm^2), identifying their atomic composition with EDXA.

Material and methods

Twenty five histological samples of lung tissue from patients (mean age (SD) 58·3 (13·7); 14 men, 12 women) with a previous pathological diagnosis of IPF were studied. All samples were obtained by surgical biopsy, mainly from the lingula. Twenty two had a pathological diagnosis of usual interstitial pneumonia (UIP), two had UIP with a desquamative component, and one patient had giant cell interstitial pneumonia. Exposure history indicated that nine patients might have inhaled inorganic particles (table

Table 1 Inhalatory antecedents in patients with idiopathic pulmonary fibrosis

Case No.	Sex	Age (y)	Antecedent*
1	M	39	No antecedent
2	W	53	No antecedent
3	M	71	Quarry 20 years
4	M	55	Cement < one year
5	W	54	No antecedent
6	W	70	No antecedent
7	M	58	No antecedent
8	W	67	Husband asbestos worker
9	W	78	No antecedent
10	M	49	Chemical industry > five years
11	W	50	Rubber factory > five years
12	W	23	No antecedent
13	M	75	No antecedent
14	W	52	No antecedent
15	M	66	No antecedent
16	M	44	No antecedent
17	M	65	No antecedent
18	M	72	No antecedent
19	M	54	Quarry > five years
20	W	31	Chemicals < one year
21	M	70	Asbestos three years
22	W	71	No antecedent
23	M	72	No antecedent
24	W	63	No antecedent
25	M	56	Asbestos 20 years

*Only antecedents that could imply inhalation of inorganic particles, even in small amounts were considered.
M = Man; W = woman.

1). Pulmonary biopsy was indicated in these patients due to a brief exposition period or because diagnoses other than pneumoconiosis were considered, which could not be confirmed without an examination of pulmonary tissue. In these patients absence of inorganic particles in the examination by optical microscopy and polarised light precluded a diagnosis of pneumoconiosis.

Twenty five samples of normal lung were also examined from patients (mean age (SD) 62·8 (14·7); 12 men, 13 women) who had died, mainly of cardiovascular causes. Patients with a history of inhalation of inorganic particles were excluded by personal history and phone call to relatives. The samples were all obtained from peripheral areas of the lung.

Samples from cases of IPF were set in paraffin and samples of normal lung were kept in formalin. Thick sections were obtained from all samples; 20 μm from those in paraffin and 1–2 mm from those in formalin. Paraffin samples were deparaffined in two baths of xylol. All samples were dried by the critical temperature method (Polaron E300) after substitution of the tissue water with acetone by immersion in baths of increasing concentration. For the SEM examination and mineralogical analysis the samples were placed on pure carbon stubs. We ruled out contamination of the stubs and paraffin with silica or silicates.

The SEM examination (Phillips SEM 500) was carried out first at low magnification (160 ×) looking for a representative area of the pulmonary parenchyma and avoiding the peribronchial, perivascular, and subpleural areas. The area selected (magnification 160 × (278 300 μm^2) was examined for inorganic particles increasing the magnification to 5000 × to identify low diameter fibres. The visualised inorganic particles were quantified and the atomic composition of each was determined using EDXA (working conditions: 25 kV; spot 0·125 μm; detector stub distance 32·5 mm; x ray angle 20°), a spectrometric technique that detects all elements with an atomic number above 10.[6 7]

The morphology and atomic composition of the particles visualised with the SEM and analysed with EDXA allows us to classify the inorganic particles as silica (detection of a peak of Si), non-fibrous silicates (Si with Mg, K, Al, Ca, or Fe), asbestos (length: width ratio > 3 ← fibrous silicate with Mg or amphibole atomic composition),[8 9] and particles of heavy elements (Fe, Pb, Ti, Ag, Sn, or Ba). The particles of elements with a possible organic origin (Ca) were not considered.

Table 2 Inorganic particles: idiopathic pulmonary fibrosis

Patient	No of particles	Type
1	0	—
2	1	Silicate (1)
3	0	—
4	0	—
5	6	Silicate (6)
6	7	Silicate (5) Silica (1) Fe (1)
7	7	Silicate (5) Silica (1) Al (1)
8	5	Silicate (3) Silica (1) ZnCu (1)
9	6	Silicate (2) Silica (2) Fe (1) Al (1)
10	4	Silicate (2) ZnCu (2)
11	0	—
12	5	Silicate (5)
13	0	—
14	6	Silicate (3) Ba (1) Co (2)
15	3	Silicate (1) Silica (2)
16	0	—
17	1	Silicate (1)
18	1	Silicate (1)
19	2	Silicate (1) Fe (1)
20	10	Silicate (4) Cu (6)
21	Innumerable	Fibrous silicate Fe (1)
22	Innumerable	Fibrous silicate
23	5	Silicate (5)
24	—	—
25	0	—

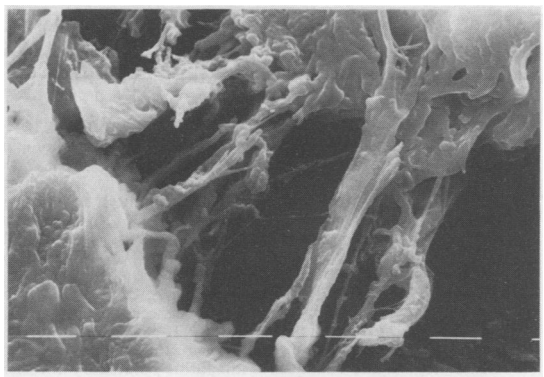

Figure 1 Asbestos fibres in a sample with a previous diagnosis of idiopathic pulmonary fibrosis (scanning electron microscopy 1250×).

Results

One sample of normal lung tissue and one sample of tissue from a patient with IPF were discarded from mineralogical analysis due to poor quality of the SEM imaging that impeded recognition of the inorganic particles. These samples were not considered in the results.

Most of the samples from patients diagnosed as having IPF contained only occasional inorganic particles (< 10 particles in the area studied), but two (8·3%) showed innumerable asbestos fibres (> 100 asbestos fibres in the area). One of these patients had an antecedent of a brief occupational exposure to asbestos. No relevant antecedent was found in the second patient (table 2; figures 1, 2).

The twenty four samples of normal lung analysed showed only occasional inorganic particles in 18/24 cases (< 10 particles). The analysis of the atomic composition of the detected particles showed a high prevalence of non-fibrous silicates (67/109 particles; 61·4%) and particles of heavy elements (38/109 particles; 34·9%). One case contained 27 inorganic particles in the area studied, and one of these particles was the only asbestos fibre found in the normal group (1/109 particles; 0·9%). This patient, together with all others in the normal group, had no relevant inhalatory antecedents (table 3).

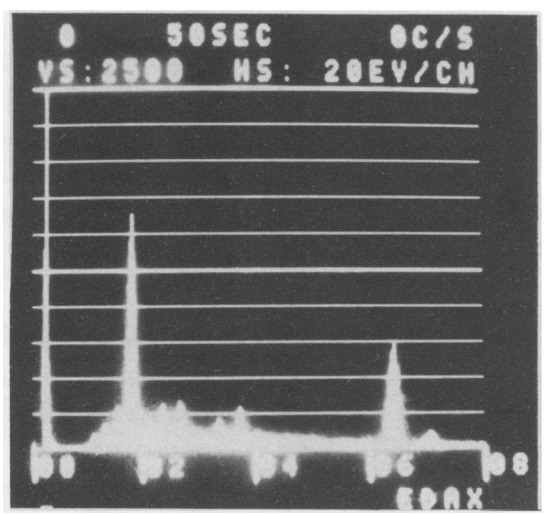

Figure 2 Energy dispersive x ray analysis of an asbestos fibre.

Discussion

Our results provide evidence that an examination using optical microscopy and polarised light overdiagnoses IPF. We found 2/24 cases with a previous diagnosis of IPF (8·3%) that contained innumerable asbestos fibres in the area studied, with no asbestos

Table 3 Inorganic particles in normal lung types

Patient	*No of Particles*	*Type*
26	7	Silicate (4) Fe (3)
27	6	Silicate (3) Fe (3)
28	6	Silicate (1) Silica (2) Fe (2) ZnCu (1)
29	5	Silicate (4) Silica (1)
30	5	Silicate (4) ZnCu (1)
31	27	Silicate (11) Asbestos (1) Pb (15)
32	3	Silicate (1) Al (1) Ag (1)
33	0	—
34	8	Silicate (8)
35	0	—
36	4	Silicate (4)
37	4	Silicate (4)
38	5	Silicate (2) Fe (3)
39	3	Silicate (3)
40	2	Silicate (2)
41	0	—
42	0	—
43	5	Silicate (5)
44	9	Silicate (7) Fe (2)
45	1	Silicate (1)
46	7	Silicate (3) Al (1) Cu (1) Fe (2)
47	1	Fe (1)
48	0	—
49	1	Fe (1)
50	—	—

bodies. The final diagnosis for these cases must be asbestosis.[10]

It has been previously suggested that IPF, or more probably a subgroup of it, could in fact be pneumoconiosis not diagnosed by standard techniques,[5 11-18] Until now this opinion has been poorly founded on original work with no control group.[15] Most of the asbestos fibres are below the 0·2 μm resolution of the optical microscope,[19] and will not be detected with a conventional examination.[20] Asbestos bodies, however, are usually easily identified and allow the diagnosis of asbestosis (two or more asbestos bodies in a pulmonary fibrosis lung sample).[21] There exists a relation between the number of asbestos bodies and the number of asbestos fibres in the lung,[22 23] but this relation is extremely variable.[24-26] It is clear, however, that the lung content of asbestos fibres is always much higher than the lung content of asbestos bodies.[24 27] This situation allows that a patient with asbestosis, sampled in an area that does not show asbestos bodies, was erroneously diagnosed as having IPF after examination with optical microscopy and polarised light.[28 29] A more sensitive analysis will correct the diagnosis.

The SEM examination of a lung sample in cases of IPF combined with the determination of the atomic composition of the inorganic particles using EDXA is easier than a mineralogical analysis using destructive techniques[30-32] and can be used as a complementary study in cases of a questionable diagnosis. Moreover, surface SEM examination offers the advantage that it can be used on samples obtained by lung biopsy that cannot be used for mineralogical analysis because there is not enough tissue available (such as our IPF lung samples).

Inorganic particles in normal lungs have not been previously studied in the western Mediterranean population. Our study shows that the particles found are mainly non-fibrous silicates (61·4%), and, rarely, silica (2·75%). We also found a high proportion of particles of heavy elements (34·9%).

It is well known that particles of silica and non-fibrous silicates can be found in normal lungs.[33] Berry *et al* studied 40 normal lungs and found Fe, Ti, and Al silicates with amorphous structure.[11] Brambrilla *et al*, examining the lungs of animals of San Diego zoo, found 95% of silicates, 5% of silica, and 5% of particles of heavy elements. The silicates were identified by *x* ray diffraction as mica.[34]

Our results allow us to conclude that standard techniques overdiagnose IPF. The use of more sensitive, non-destructive techniques such as SEM and EDXA in diagnosis of IPF could show that some of the lung samples with a previous diagnosis of IPF are really asbestosis.

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